Prevention of Dental Caries-Measures beyond Fluoride

Ravi Agarwal*, Chanchal Singh, Ramakrishna Yeluri and Kalpna Chaudhry
Department of Pedodontics, K D Dental College and Hospital, Mathura, India

Abstract
Dental caries is one of the most common infectious microbial diseases. It is rightly said that prevention is better than cure. Various steps in prevention of dental caries have been taken, fluoride being the most common among them. Various non-fluoride agents are also present which helps in prevention of dental caries. Various agents like, arginine, plant extracts, sucrose free polyol gums, probiotics, novamin, Dentifrices, antimicrobials, CPP-ACP, probiotics, novamin, Dentifrices, antimicrobials, etc are commonly used in prevention of dental caries. This paper reviews about these non-fluoride agents in the prevention of dental caries.

Keywords: Dental Caries; Non-fluoride agents; Prevention

Introduction
Dental caries is one of the most common preventable childhood disease; people are susceptible to this ailment throughout the lifetime [1]. Data from the National Health and Nutrition Examination Survey (NHANES) conducted between 1999 and 2004 revealed that 28 percent of children ranging from 2 to 5 years of age had one or more primary tooth affected by dental caries and 51 percent of children had one or more primary tooth affected by age 6 to 11. In the permanent dentition, 10 percent of children aged 6 to 8 had dental caries and 51 percent of children were affected by age 12 to 15 [2]. The preventive care providers have the intention for prevention beyond the scope of providing hygiene therapy and oral hygiene instructions. Dental decay is mainly due to demineralization which is caused by acids produced by bacteria, particularly mutans Streptococci and possibly lactobacilli that ferment dietary carbohydrates. This occurs within a bacteria-laden gelatinous material called dental plaque that adheres to tooth surfaces and becomes colonized by bacteria. Thus, caries results from the interplay of three main factors over time: dietary carbohydrates, cariogenic bacteria within dental plaque, and susceptible hard tooth surfaces [3]. The use of fluoridated toothpastes [4], other topically applied fluorides [5], fluoridated municipal water [6] and pit and fissure sealants [7,8] along with dietary improvement remain mainstays of caries management. These modalities, which are based on high quality evidence, are the first choice for prevention and control of dental caries. Fluoride’s anti-caries efficacy is well-proven and may arise from multiple modes of action, i.e., inhibition of tooth demineralization, promotion of incipient lesion remineralization, and perhaps, antibacterial effects on cariogenic bacteria [9]. Clinical evidence would suggest that increasing the concentration of fluoride above the conventional level of 1000 or 1500 ppm in dentifrices will give an increased benefit although there would appear to be something of a law of diminishing returns [10]. Although fluoride is highly effective on smooth-surface caries, its effect would seem to be more limited on pit and fissure caries, and these lesions tend to dominate the caries experience of developed countries currently enjoying the benefits of fluoride. Apart from fluoride, dental health education programme, diet counselling, oral hygiene measures like dentifrices, different brushing technique are also used in prevention of dental caries. Various anti plaque agents, and other agents like enzymes have been effectively used as prevention of dental caries. From the 1930’s when the researches came to know the anti-caries effect of the fluoride, many gargantuan water fluoridation program have been implicated, but these programs has shown to be successful only in attaining a 50% caries reduction but none of them was able to contain the caries process [11]. Since 1970’s researches started to search for non-fluoride agents for the prevention of dental caries [12]. Non-fluoride agents may serve as adjunctive therapeutics for preventing, arresting or even reversing dental caries. The objective of this paper is to present a complete review of recent advances of various non-fluoridated caries preventing agents.

Recent Advances in Caries Prevention

Arginine
Arginine, a common amino acid found in saliva is broken down by oral plaque bacteria to acid neutralizing alkali. The production of acid by dental plaque is the direct cause of dental caries; it is noteworthy that increases in the proportions of aciduric organisms appear to occur at the expense of species that are less aciduric and generally associated with dental health; including Streptococcus sanguinis and Streptococcus gordoni [13-15]. Some of the less aciduric organisms associated with dental health derive protection from plaque acidification by hydrolysing urea or arginine to ammonia, either by expressing a urease enzyme or by the arginine deiminase system (ADS), respectively. Production of ammonia by oral bacteria can positively influence the balance between remineralization and demineralization of the tooth and may help to prevent the emergence of a cariogenic microflora [16-18]. Therefore, the capacity of oral biofilms to generate alkali appears to be a major caries-inhibiting factor [19]. Urea and arginine can be rapidly metabolized by oral bacteria to elicit a rise in environmental pH. A strong correlation between elevated levels of free arginine in saliva and caries resistance has also been revealed [20]. In addition, dental plaque of caries-resistant individuals has been shown to have higher pH values compared to the plaque of caries-susceptible individuals, and in part the increased pH has been correlated with elevated ammonia levels [21-33].
Plant extracts

There is a global need for alternative prevention and treatment options and products for oral diseases that are safe, effective and economical. One such strategy would be to verify the enormous use of medicinal plants. A number of phytochemicals, including antibacterial agents have been derived from edible plants and demonstrate antibacterial properties against *Streptococcus mutans*.

Neem, *Azadirachta indica*: Wolinsky et al. [24] investigated the inhibitory effects of acqueous extracts from Neem upon bacterial aggregation, growth and adhesion to hydroxyapatite and production of insoluble glucan, which may effect in *in vitro* plaque formation.

**Tulsi, Ocimum sanctum:** Tulsi, *Ocimum sanctum* is a plant of Indian origin and a time tested premier medicinal herb. The extract of tulsi is used to treat a variety of illnesses that include diabetes mellitus, arthritis, bronchitis and skin diseases. The antimicrobial property of tulsi has been tested against a variety of microorganisms like *Staphylococcus aureus*, *Klebsiella*, *candida albicans*, *E. coli* and *proteus sp.* The antimicrobial activity of tulsi is attributed to its constituents namely ursolic acid and carvacrol. Agarwal et al. [25] in their study demonstrated an antimicrobial potential of tulsi extract at various concentrations and achieved maximum antimicrobial potential at 4% concentration level.

**Prunus mume:** *Prunus mume* is a common fruit in Asia, which has been used in traditional Chinese medicine. It is considered to be the potential candidate for developing an oral antimicrobial agent to control or prevent dental diseases associated with oral pathogenic bacteria like *Streptococcus mutans*, *S. sobrinus*, *S. mitis*, *S. Sanguinis*, *Lactobacillus acidophilus*, *P. gingivalis*, Aggregatibacter actinomycetem comitans [26].

**Green and black tea (Camellia sinensis):** Various component in green and black tea (leaves of Camellia sinensis, [Theaceae]) notably simple catechins, have antiaggregating action. These include: a direct bactericidal effect against *S. mutans* and *S. sobrinus*; prevention of bacterial adherence to teeth; inhibition of glucosyl transferase, thus limiting the biosynthesis of sticky glucan; inhibition of human and bacterial amylases. Ferrazzano et al. [27] concluded that the antiaggregating effect against alfa hemolytic streptococci by polyphenols from cocoa, coffee and tea suggest further possible application of these beverages in the prevention and pathogenesis of dental caries.

**Hop plant (Humulus lupulus):** Tagashira et al. [28] reported the inhibition of *S. Mutans* and other oral streptococci, by the antimicrobially active ingredients of hop plant. They found that all tested hop constituents inhibited the streptococci with minimum inhibitory concentration at pH 7.5 ranged from 2 to 50 µg/ml. Antimicrobial activity of hop constituents was greater than other plant products such as thymol, neroil, cinnamon oil, oil of clove, menthol and eucalyptol.

**Oleic acid, Linoleic acid, epicatechin polymer (Cacao bean husk):** These shows antimicrobial activity against planktonic cells of mutants Streptococci. It has an inhibitory effect on water-insoluble substances, polymer glucan synthesis, adherence, acid production by mutants streptococci. It also helps in reduction in plaque accumulation and caries development in rats infected with *S. mutans or S. sobrinus* [29,30].

**Proanthocyanidins, phenolic acids, flavonols (Cranberry):** These shows antimicrobial activity against biofilm cells of mutants streptococci. It causes disruption of acidogenic/aciduric properties of planktonic and biofilm cells of *S. mutans*. It has inhibitory effects on Gtf activity and adherence by mutants Streptococci and causes reduction of formation of *S. mutans* biofilms and EPS content. Reduction in caries development in rats infected with *S. mutans* is also seen [31-33].

**Apigenin and tt Farnesol:** Apigenin and tt Farnesol are two naturally occurring agents that affect the development of cariogenic biofilms. Apigenin inhibits the activity of glucosyntransferases in solution and on the surface of saliva-coated hydroxyapatite beads and it was devoid of antibacterial activity. tt-Farnesol showed modest antibacterial activity against biofilms and its effects on glucosyltransferases were minimal [34]. It also enhances the cariostatic effectiveness of fluoride. The combination of these novel agents with fluoride may represent a potentially useful and an alternative approach to the current chemotherapeutic strategies to prevent this ubiquitous disease by reducing the expression of virulence of *S. mutans* without necessarily suppressing the resident oral flora [35].

**Meswak chewing sticks (Twigs of Salvadora persica):** These sticks embedded in agar or suspended above the agar plate had strong antibacterial effects against all tested bacteria. The antibacterial effect of suspended meswak sticks suggested the presence of volatile active antibacterial compound [36-39].

**Propolis:** Propolis is a natural beehive product, and cacao bean husk extracts have also shown significant antibacterial activity against *S. mutans* and/or *S. sobrinus in vitro* [29,38,39] Propolis extract when used as a mouthwash exhibits an *in vivo* antimicrobial activity against *S. mutans* and might be used as an alternative measure to prevent dental caries [40]. Topical applications of chemically characterized Propolis extracts have also been shown to be highly effective in reducing the incidence and severity of smooth surface and sulcal caries in rats [39,41]. However, the cariostatic effects of propolis are highly variable depending on its chemical composition and geographical origin. There are many other reports in the literature concerning the antimicrobial activities that various plant extracts may have against cariogenic bacteria, although the majority of these studies provide limited or incomplete information due to the lack of chemical characterization of the extracts. However, there are a few exceptions. For example, Li et al. [42] have identified gallotannins from Melaphis chinensis and triterpenes (oleanolic acid and oleanonic acid) from *Ceanothus americanus* as antimicrobial agents that harbor activity against mutants streptococci. Furthermore, a chemically characterized extract of *Galla chinensis* (containing gallic acid and methyl gallate) has been demonstrated to impede the growth of *S. mutans* and other caries-related organisms, including *Lactobacillus rhamnosus* and *Actinomyces naeslundii*, within biofilms [43]. Recently, established that naturally occurring phenolic compounds generally display antibacterial activity by disrupting the membrane lipid-protein interface as nonionic surface-active agents [44]. Ramakrishna et al. [38] studied various natural alternatives derived from plants and plant products and concluded that it can serve as a prevention and treatment option against cariogenic bacteria.

**Chinese Licorice Root:** A new cavity fighting herbal lollipop that contains a special herbal formula extracted from the Chinese licorice root can help to immobilize major organizations responsible for tooth decay. These orange flavoured herbal lollipops was discovered by microbiologist at the UCLA school of dentistry should be consumed twice a day- one in the morning after breakfast and another after professional teeth cleaning between two and four times a year.

**Xylitol:** A sweet alternative discovered in 1891 by German chemist
Emil Fischer. Dental benefits of xylitol were first recognized in Finland in 1970 using animal models. The first chewing gum developed with the aim of reducing caries and improving oral health was released in Finland in 1975 and in United States shortly after. Xylitol is not fermented by cariogenic plaque bacteria and thus does not lower the pH of the plaque. It reduces the accumulation of plaque on the surface of the tooth. It accumulates intracellular in MS and inhibits the bacterial growth. Xylitol reduces MS by altering their metabolic pathways. It has long been known to have antibacterial properties, in particular to depress the proportion of Streptococcus mutans in plaque [45,46], and also to reduce its acidogenicity [47]. In addition, xylitol has been suggested to have an ability to inhibit enamel dissolution in vitro [48]. In clinical studies, xylitol chewing gums have, in general, been reported to inhibit the development of caries [49]. In children reporting caries experience, consumption of xylitol containing lozenges or hard candy reduces incidence of coronal caries [50-52]. For children below age two, in addition to the study that evaluated xylitol tablets, the xylitol-containing syrup among children in the Marshall Islands and reported a statistically significant difference in favor of xylitol syrup [53]. There is insufficient evidence that xylitol syrup prevents caries in children under 2 years of age. There is insufficient evidence regarding xylitol dentifrice as it contains other ingredients which might inhibit the production of dental caries [54,55].

**Probiotics and replacement therapy:** Probiotics are defined as live micro-organisms, principally bacteria, that are safe for human consumption and when ingested in sufficient quantities, have beneficial effect on human health, beyond basic nutrition [56]. First probiotic species to be introduced in research was Lactobacillus acidophilus by Hull et al. in 1984; followed by Bifidobacterium bifidum by Caglar et al. [57]. Probiotic have shown to influence immune system through several molecular mechanisms: In oral cavity, probiotics can create a biofilm, acting as a protective lining for oral tissues against oral diseases. Such a biofilm keeps bacterial pathogens off oral tissues by filling a space pathogens would invade in the absence of the biofilm; and competing with cariogenic bacteria and periodontal pathogens growth. The administration of probiotic lactobacilli (LGG) in milk to kindergarten children in Helsinki, Finland resulted in reduction of initial caries development. Cornelli et al. [58] studied 23 dairy bacterial strains for the prevention of dental caries and reported that only two strains namely Streptococcus thermophilus and Lactococcus lactis were able to adhere to saliva-coated hydroxyapatite and were further successfully incorporated into a biofilm similar to the dental plaque. Recently it was shown that probiotic cheese reduced the prevalence of oral candida [59]. Cheese might be the ideal vehicle for administering probiotics to humans. Cheese enhances remineralization and prevents demineralization of enamel. Yogurt products containing L. reuteri showed a significant growth inhibitory effect against S. mutans, while yoghurts with lactobacilli other than L. reuteri did not show such inhibition. Residence time of probiotics in oral cavity after treatment withdrawal was studied by Çağlar et al. [60]. A reduced S. mutans level was shown after a two-week use of a L. reuteri-enriched yogurt; effects were observed during use and for a few days after discontinuation. A loss of L. reuteri colonization was observed by Wolf et al. [61] two months after having discontinued probiotic use. Sucking a medical device containing the probiotic lozenge with L. reuteri once daily for 10 days reduced the levels of salivary mutants [62]. However it is unlikely that a permanent colonization occurs. Therefore, regular consumption of probiotic products is needed to maintain the preventive and therapeutic levels.

In addition to probiotics, another measure that competitively reduces the pathogen composition in the oral flora has emerged with the advances in gene engineering and DNA recombination technology. This method is the so-called replacement therapy. Replacement therapy involves the use of a harmless effector strain that is permanently colonized in the host’s microflora. This effector strain is designed to prevent the colonization or outgrowth of a particular pathogen. To prevent an infection using replacement therapy (recently referred to as probiotic therapy), a natural or genetically modified effector strain is used to intentionally colonize the sites in susceptible host tissues that are normally colonized by a pathogen. If the effector strain is better adapted than the pathogen, colonization or outgrowth of the pathogen will be prevented by blocking the attachment sites, by competing for essential nutrients, or via other mechanisms. As long as the effector strain persists as a resident of the indigenous flora, the host is protected potentially for an unlimited period of time. S. mutans strain BCS3-L1 is a genetically modified effector strain designed for use in replacement therapy to prevent dental caries. To be an effective effector strain, BCS3-L1 must satisfy four prerequisites: It must have a significantly reduced pathogenic potential to promote caries. It must persistently colonize the S. mutans sites, thereby preventing colonization by disease-causing strains whenever the host comes into contact with it. It must aggressively displace indigenous strains of S. mutans and allow previously infected subjects to be treated with replacement therapy. It must be safe and not make the host susceptible to other disease conditions. From a standpoint of replacement therapy for caries prevention, implantation of an effector strain would best be achieved in children immediately after tooth eruption and before the acquisition of a caries-inducing strain. A final aspect of replacement therapy safety is the requirement for controlled spread of the effector strain within the population. Mutacin 1140 up-production clearly provides a selective advantage to BCS3-L1 colonization. However, the minimum infectious dose has not been determined for this strain or any S. mutans strain in humans.

**Novamin:** Novamin falls into a new category of bioactive glass-ceramic material that has been available since the 1960’s as materials to help in bone repair. The active ingredient is a calcium sodium phosphosilicate that reacts when exposed to aqueous media, thus providing calcium and phosphate ions to the applied surface. Examples of Novamin powered technology include Oravive, a product from Natural Health Organics, which is a non-fluoridated, and non-prescription dentifrice containing 5% Novamin. Bioactive glasses have been tested under different clinical situations, such as having an antibacterial effect [63]. Novamin-containing dentifrice is statistically more effective than a placebo dentifrice [64]. One of these studies was done by and compared a dentifrice containing 5% Novamin and Fluoride (MFP) to a commercially available dentifrice in remineralization of subsurface carious lesions in human tooth enamel. It used confocal laser scanning microscopy (CLSM), which is able to distinguish between sound enamel and demineralized enamel using a fluorescent dye [65].

**Dentifrices:** Tooth-pastes are the valuable adjuncts to oral hygiene as they make brushing more pleasant and more effective. Many attempts have been made at various times to add-therapeutical agents with the object of interfering with oral flora, limiting plaque formation and making teeth more resistant to caries.

**Chlorophyll:** Chlorophyll was one of the earliest agents added to the paste and is still present in some tooth-pastes. Although *in vitro* tests showed that chlorophyll- containing tooth pastes limits bacterial growth, but clinical trials have not shown any anti- caries effects [12,66].
Ammoniated tooth-paste: This usually contains urea, and developed in an attempt to control the acid production in plaque. A numbers of clinical trials were carried out, but all gave very little positive or inconclusive results. Ammoniated pastes have been superseded by more effective agents, Anti-biotic toothpastes containing penicillin, triclosan or topical anti-biotic such as tetrathrinic have also been tried. It was based on the assumption that if acidogenic bacteria are destroyed, caries will be controlled.

Anti-enzyme paste: These toothpastes were introduced on the basis that they interfere with enzyme systems of the bacteria and thus with their growth and function. Still their effectiveness has not been evaluated by clinical trials [12,66]. Various other dentrifices containing herbal products like neem, tulsi, clove oil, propolis are available which show beneficiary effect in preventing dental caries.

Antimicrobials: Typically antimicrobial agents target both supra-gingival plaque, and more importantly sub-gingival plaque buildup. Clinical decisions to deliver antimicrobial agents exist in a balance between delivering relevant and clinically amounts, but at the same time not disrupting the natural ecology of the mouth which provides protection from opportunistic pathogens and the overgrowth of exogenous microbes.

Chlorhexidine: Currently mouthrinses that contain 0.12% chlorhexidine are marketed within the United States. Chlorhexidine is a broad spectrum antibiotic that kills Gram-positive and Gram-negative bacteria as well as yeasts at high concentrations. At lethal concentrations chlorhexidine causes irreparable damage to the cell membrane of target microbes, and at sub-lethal concentrations chlorhexidine can interfere with the sugar transport and acid production of the cariogenic streptococci strains, providing a bacteriostatic effect. Chlorhexidine is typically utilized because of its great retention within the plaque coated enamel surface, and studies report that 30% of the delivered chlorhexidine is retained in the mouth after use [67,68]. The efficacy of the chemical has come under scrutiny as some clinical trials have failed to produce significant level of caries reduction, and side effects such as staining and an altered taste sensation have been reported [69].

Triclosan is used to increase the ability of mouthwashes to bind to the oral mucosa, and thus be available for long period of time. Jenkins et al. compared the magnitude and duration of salivary bacterial count reduction produced by a single rinse of 0.2% triclosan, 1% sodium lauryl sulfate and 0.2% chlorhexidine mouthwashes. They found considerable reduction in bacterial count which remained significant for three hours with triclosan and for 7 hours with sodium lauryl sulfate and chlorhexidine [70]. The use of 0.3% triclosan mouth rinse showed significant reduction in salivary Streptococcus mutans count [71].

Essential oils: Essential oils have also been extensively studied for antimicrobial activity against caries-related bacteria. Essential oils derived from plants are typically a complex mixture of approximately 20-60 compounds that are in solution at various concentrations. Overall, the main chemical group is primarily composed of terpenoids, followed by aromatic and aliphatic congeners [72]. Thymol and eugenol inhibit the growth of a wide range of oral microorganisms including mutans streptococci [36,37].

Trace elements: Different trace elements has been investigated were zinc, tin, aluminium, copper, iron, strontium, barium, manganese and molybdenum, gold, lead etc. Aluminium, copper, and iron have the most commonly used as cariostatic agent, although each would probably have organo-leptic problems if used in oral care products as simple salts. Moreover, the toxicity of many metals like aluminum, copper, barium molybdenum, would restrict the concentration at which they could be safely used [66,73].

CPP-ACP: Recent developments in the area of remineralization include casein phosphopeptide-amorphous calcium phosphate (CPP-ACP) Dairy products such as milk, milk concentrates and cheese are recognized as non-cariogenic or cariostatic in several laboratory studies due to the presence of milk phosphoprotein, casein [74]. The casein phosphopeptides (CPP) are derived from casein by trypsin digestion. In 1987, Reynolds found that CPPs were incorporated into the intra-oral appliance plaque and were associated with a substantial increase in the plaque’s content of calcium and phosphate [75]. All CPPs contain the sequence motif -Pse-Pse-Pse-Glu-Glu-, where Pse is a phosphoserine residue. Through these multiple phosphoserine residues, CPPs have a marked ability to stabilize calcium phosphate ions in solution and to form an amorphous calcium phosphate (ACP) complex, referred to as CPP-ACP [74,76]. The milk protein, CPP, stabilizes high concentrations of calcium phosphate ions in ACP solutions. The CPP-ACP is taken up by dental biofilms and localizes to the enamel surface as nanoparticles. Calcium, phosphate and fluoride from CPP-ACP, which are released during Acidogenic challenge, help to maintain the supersaturated state of these ions in the biofilm and so promote remineralization over demineralization [77]. Several randomized clinical trials (RCT) have shown that CPP-ACP added to sugar-free chewing gums, [78] tooth paste [79] or dental cream [80,81] increased enamel subsurface remineralization. These RCT results suggested both a short-term remineralization effect of CPP-ACP and a caries-preventing effect for long-term clinical CPP-ACP use [82]. A recent study has demonstrated that CPP could be detected on the tooth surface 3hours after chewing sugar free gum containing CPP ACP. Recaldent is an active ingredient derived from casein, part of protein found in cow’s milk. It works safely; strengthen teeth by delivering calcium and phosphate in a unique soluble form to remineralize enamel. Recaldent will not affect people with lactose intolerance. The acid resistance of enamel lesions remineralization in situ by a sugar free chewing gum containing CPP ACP is similar to gum not containing CPP ACP [83]. Cai et al. [84] demonstrated the effect of CPP ACP incorporated into a sugar free lozenges on enamel remineralization in a human in situ model.

Conclusion
We have a variety of new agents which can be used to prevent dental caries but application of these agents in clinical trials is still limited in the developing countries. Moreover dental caries is a multifactorial and all non-fluoride measures should be evaluated properly in human trials so that they can be introduced at the community level for the prevention of dental caries.

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